ORIGINAL ARTICLES AAEM

Ann Agric Environ Med 2004, 11, 35–44

ENVIRONMENTAL FACTORS AFFECTING THE START OF POLLEN SEASON AND CONCENTRATIONS OF AIRBORNE *ALNUS* POLLEN IN TWO LOCALITIES OF GALICIA (NW SPAIN)

Francisco Javier Rodríguez-Rajo[1], Angeles Dopazo[2], Victoria Jato[1]

[1]Department of Plant Biology and Soil Sciences, Sciences Faculty of Ourense, University of Vigo, Ourense, Spain
[2]Department of Plant Biology, Faculty of Pharmacy, University of Santiago de Compostela, Santiago de Compostela, Spain

Rodríguez-Rajo FJ, Dopazo A, Jato V: Environmental factors affecting the start of pollen season and concentrations of airborne *Alnus* pollen in two localities of Galicia (NW Spain). *Ann Agric Environ Med* 2004, **11**, 35–44.

Abstract: *Alnus* pollen is an early component of the annual atmospheric aerosol of the north-west regions of Spain, which causes the first occurrence of allergic symptoms. Seasonal and intra-daily variation of *Alnus* pollination, and the influence that main meteorological parameters exert, was studied in this paper. Monitoring was carried out from 1993–2002, by using two Lanzoni VPPS 2000 volumetric samplers. Once the atmospheric behaviour of this pollen had been identified, the final objective was to elaborate predictive models to determine the onset of the *Alnus* pollen season and its concentrations during the pollination period in two localities of north-west Spain (Santiago and Ourense). Winter chilling required to overcome the bud-dormancy period was similar in both cities, with around 800 Chilling Hours (C.H.) and 5.5ºC threshold temperature. Calculation of heat requirement for bud growth was carried out with maximum temperature, with around 50 Growth Degree Days (G.D.D.ºC) needed, with 6ºC threshold temperature. Data from 2002 were used in order to determine the real validity of the models. This year was not taken into account to establish the aforementioned models. The variation between the predicted start of the pollen season and the observed season was smallest in Ourense. Verifying the proposed models for predicting daily mean concentrations of *Alnus* pollen during the pollen season shows that the predicted curves fits the observed variations of daily mean concentrations.

Address for correspondence: Dr. F. Javier Rodríguez Rajo, University of Vigo, Sciences Faculty of Ourense, Department of Plant Biology and Soil Sciences, Ourense, 32004, Spain. E-mail: javirajo@uvigo.es

Key words: ***Alnus*, Spain, pollen, aerobiology, predictive models.**

INTRODUCTION

The study of the pollen-season timing of species located at their biogeographical distribution limit allows us to explain changes in ecology related to the possible global climatic change. This is the case of the *Alnus glutinosa* (L.) Gaertner, a tree broadly represented in the north-west of Spain, where it mainly forms part of riverside forests. Atmospheric alder pollen is a common winter fraction of the bioaerosol in this region with a special aerobiological interest due to its capacity to cause annual early hay fever symptoms. The allergenic importance of its pollen grain has led to a great number of papers centred on the study of its allergens [14, 26]. In the regions of Central Europe and sub-Atlantic areas characterized by warm winters, alder pollen determines the early manifestation of pollinosis [14, 41]. Studies carried out by different authors point out that in north-west Spain between 9% and 20% of hay fever sufferers are allergic to *Alnus* pollen [4, 7, 13, 18]. Although its

Received: 4 November 2003
Accepted: 28 February 2004

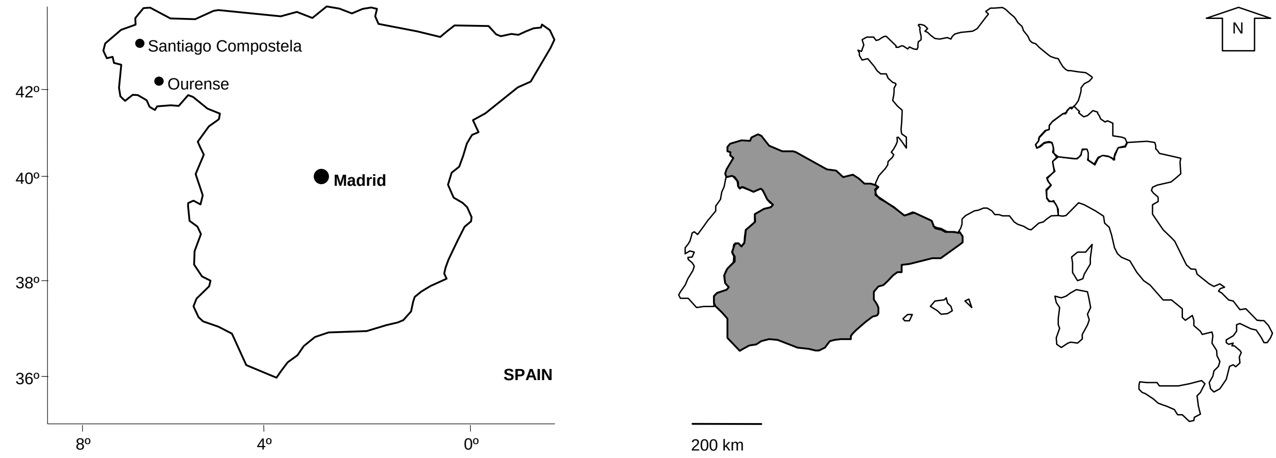

Figure 1. Location of the studied localities in Spain.

sensitisation is quite frequent, its epidemiology is not very important since this pollen type appears in the atmosphere in periods of the year in which, because of the climate, exposure to its allergens is not carried out over a long period of time [50]. It has a high degree of crossed reactivity with pollen grains of other genus of the Betulaceae family [14] and even with some of the Fagaceae family [28].

In recent years, one of the most important aims in Aerobiological studies has been to find forecast models that enable us to know both the start date and the severity of the pollen season. This knowledge facilitates the beginning of preventive treatments several days before the start of pollination, and optimises their effectiveness.

The woody plants of temperate regions have evolved mechanisms during the periods of adverse meteorological conditions in order to preserve their cells from risk of frost. The strategy involves a requirement for winter chilling followed by another of warm temperatures [52], to break a physiologic state of inactivity denominated by dormancy. The quantification of chilling and heat requirements to overcome this period of dormancy enables us to determine the onset of pollination, which is of great importance to allergic individuals [16, 23, 30, 44]. During flowering, meteorological factors such as hours of sunshine, temperature, rainfall and relative humidity determine the opening of the anthers, while wind finally determines the dispersion of pollen and other particles of the aerosol into the atmosphere [9, 20]. In general, pollen-predictor models are based on the correlation of pollen levels with the aforementioned meteorological variables [33], developing linear regression equations with greater prediction capacity [1, 30, 40, 41, 49].

The objectives of this study are the identification of seasonal and intra-daily variations of the atmospheric concentrations of *Alnus* pollen and the main influence of meteorological parameters on the aerobiology of this pollen. Finally, prediction models are established to determine the onset of *Alnus* pollen season and its concentrations during its pollination period in two localities in north-west Spain.

MATERIAL AND METHODS

The cities of Ourense and Santiago of Compostela are located in north-west Spain (Fig. 1). Ourense has an ombrothermic dry and warm climate, with an annual mean temperature of 14ºC, an average minimum temperature of 9.2ºC, an average maximum temperature of 18.9ºC and total annual precipitation of 772 mm [31]. The city of Santiago of Compostela has a temperate sub-dry climate, with an annual mean temperature of 12.9ºC, an average minimum temperature of 8.8ºC, an average maximum temperature of 17.1ºC. Its total annual rainfall is 1,288 mm [34, 10].

In biogeographical terms, Santiago of Compostela is located in the Euro-Siberian region, Cantabrian-Atlantic region, while Ourense is located in the Mediterranean region, Carpetan-Iberian-Leonese region. The genus *Alnus* is represented in the study area by the single species *Alnus glutinosa* (L.) Gaertner. In a natural way, this tree is associated with rivers and valley floors, where the dominant formations are alder groves characterized by *Alnus glutinosa* (L.) Gaertner, with frequently appearing *Betula alba* L., *Frangula alnus* Miller, *Fraxinus angustifolia* Vahl., and *Salix atrocinerea* Brot [45].

Pollen sampling was undertaken by means of two LANZONI VPPS 2000 volumetric traps [27], located in Ourense on the roof of the Sciences Faculty and in Santiago on the roof of the Student Information Centre (COI) of the South Campus. The samplers were placed 25 and 15 m above ground level respectively. The daily average alder pollen counts were recorded continuously from 1993–2002 inclusive.

The atmospheric pollen grains were counted following the model proposed by the Spanish Aerobiological Network (R.E.A.), based on four longitudinal transects along the slides [12]. Methods described by different authors [2, 22, 38] were taken into account to obtain the main pollen season, being selected the first of such since it had the lowest standard deviation coefficient. The pollination period was calculated as 95% of the annual total pollen-production season; the first days, producing up to 2.5% of total production, were removed from the calculations as well as the final days (from 97.5% to 100.0% of total production).

In order to obtain a model reflecting the fluctuation of the pollen concentration throughout the day, we followed the model which selects the days on which the pollen concentration is greater than the mean of the main pollen season and, within this period, the days without rainfall [21]. The resulting days were used to calculate the mean concentration every two hours, thereafter expressing the data as percentages.

Spearman's correlation test was used to find a possible relationship between *Alnus* pollen concentrations and the main meteorological factors: rainfall (mm), relative humidity (%), hours of sunshine (hours), maximum, minimum and mean temperatures (ºC), thermal oscillation as the difference between the maximum and minimum daily temperatures (ºC), wind direction (%) and wind speed (m/s). Furthermore, in the case of temperatures, thermal oscillation and hours of sunshine, we used their accumulated sums corresponding to one and two days. The pre-peak period was selected, established as the period from the first day of the pollen season until the day of maximum pollen counts. Weather data were supplied by the National Institute of Meteorology, from a station located at 100 m. of the sampling point.

To predict the date of the beginning of *Alnus* pollination, the quantification of chilling and heat requirements to overcome the dormancy period were considered. In the present study, to determine chilling requirement we chose a thermal time model [5]. In the present paper we tested the accumulation of chilling hours between 0–7.2ºC, and nine different threshold temperatures (5.5-6-6.5-7-7.5-8-9-10-11ºC):

$$C.H. = 801 + 0.2523\,B + 7.57\,B^2 \times 10^{-4} - 6.51\,B^4 \times 10^{-10} - 11.44\,Ti_{min} - 3.32\,Ti_{max}.$$

C.H. = number of Chilling Hours during the period.

$$B = 24\,D\,((Threshold - T_{min})/(T_{max} - T_{min}))$$

Where Ti_{min} and Ti_{max} are respectively the average minimum and maximum temperatures during the period considered, D is the number of the days of the same period, and *Threshold* the threshold temperature considered.

We used this model selecting the period with two different criteria. The first, taking into account the period from the day when the daily mean temperature decreased below the threshold temperature, to the day (in the first half of January) when the minimum was recorded and a change in temperature trend was recorded [5]. The second, selecting the days in which meteorological conditions did not allow any physiological activity in the tree during the same period [31]. This was the case when the daily maximum temperature was equal to or less than the threshold temperature [36].

Heat requirement (H.R.) expressed as Growth Degree Days (G.D.D.ºC) was estimated as a function of the sum of the daily maximum temperatures from the end of the chilling period to the beginning of the pollen season, taking into account different threshold temperatures (6-6.5-7-7.5-8ºC): $H.R. = \Sigma(T_{max} - threshold)$ [48].

The lowest standard deviation and variation coefficient were used to identify the most adequate threshold temperature, in both the chilling and forcing-temperature period.

Finally, we tried to predict pollen concentrations by means of linear regression studies, using as pollen concentration estimators the meteorological variables with the highest positive correlation coefficients.

Table 1. Characteristics of *Alnus* pollen season calculated according 2.5% method [2].

	1993	1994	1995	1996	1997	1998	1999	2000	2001	1993-2001
Ourense										
Start of pollen season	08-Jan	29-Dec	10-Jan	03-Jan	05-Jan	07-Jan	12-Jan	11-Jan	01-Jan	06-Jan
End of pollen season	08-Mar	19-Feb	21-Feb	04-Mar	12-Feb	23-Feb	27-Feb	15-Mar	19-Feb	25-Feb
Length (days)	63	57	43	62	39	48	47	65	50	53
Maximum (pollen/m^3)	75	163	175	91	534	72	161	113	94	164
Date of the maximum	16-Jan	15-Jan	25-Jan	03-Jan	27-Jan	21-Jan	01-Feb	30-Jan	08-Jan	18-Jan
Total Annual	1478	1153	1186	1448	3257	849	1438	699	661	1352
% Total pollen	6.4	5.1	5.5	7.1	15.4	4.7	10.4	4.1	4.5	7.0
Nº Days >30 pollen/m^3	19	9	9	18	23	11	12	5	7	13
Nº Days >80 pollen/m^3	0	2	6	1	12	0	8	2	3	4
Santiago										
Start of pollen season	06-Jan	12-Jan	09-Jan	17-Jan	14-Jan	10-Jan	15-Jan	25-Jan	19-Dec	10-Jan
End of pollen season	03-Mar	23-Feb	17-Feb	12-Mar	08-Mar	03-Mar	23-Feb	28-Mar	27-Feb	03-Mar
Length (days)	57	43	68	56	54	53	40	35	71	53
Maximum (pollen/m^3)	67	232	44	12	158	91	92	56	33	87
Date of the maximum	09-Feb	30-Jan	16-Feb	14-Feb	10-Feb	10-Feb	31-Jan	31-Jan	08-Jan	03-Feb
Total Annual	904	1438	424	179	1169	794	835	278	230	695
% Total pollen	4.4	7.2	2.7	3.8	4.7	4.5	3.9	2.2	1.2	3.8
Nº Days >30 pollen/m^3	8	16	4	0	14	8	9	3	1	7
Nº Days >80 pollen/m^3	0	3	0	0	4	1	2	0	0	1

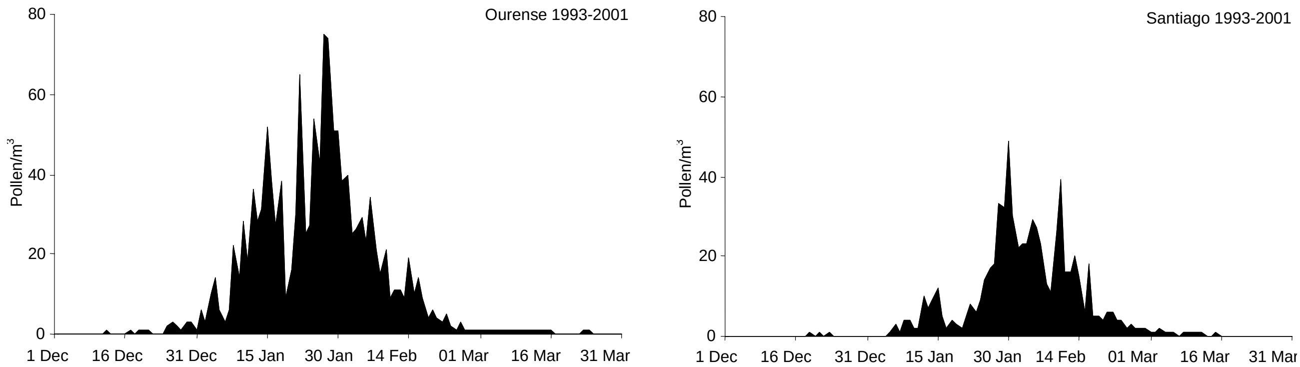

Figure 2. Average pollen concentrations of Ourense and Santiago de Compostela during the period studied.

We applied Scheffé's test to study the homogeneity of the populations under study, which would enabled us to identify the presence of anomalous years, which were out of line with the general behaviour and could produce alterations in the proposed prediction models. The analysis was carried out taking into account the different meteorological parameters throughout the years under study.

RESULTS

The pollen of *Alnus* is one of the first to appear in the annual spectrum of the studied localities. It is present in the atmosphere during the winter months, mainly in January and February. The pollen season has an average duration of 53 days in both towns (Tab. 1). Generally, its onset takes place during the first fortnight in January, with an average date of 6 January and 10 January in Ourense and Santiago respectively.

The quantity of total pollen was highly variable, oscillating between 3,257–661 pollen grains in Ourense and between 1,438–230 in Santiago. In Ourense, 1997 was the year with the highest annual total while in Santiago it was 1994. The annual mean value for both localities was 1,352 and 695 pollen grains in Ourense and Santiago respectively. The percentage of *Alnus* pollen with respect to total annual pollen also fluctuated extensively: between 15.4–4.1% in Ourense and 7.2–1.2% in Santiago. Although this pollen type only includes a single species, the curve of daily mean values is characterised by several concentration peaks throughout the pollen season (Fig. 2).

The maximum concentration values were generally attained during the second fortnight in January in Ourense and some days later in Santiago. The highest value recorded in Ourense was 534 pollen/m^3 on 27 January, 1997, while in Santiago it was 232 pollen/m^3 on 30 January, 1994. The number of days on which the pollen of *Alnus* was present in the atmosphere in concentrations higher than 30 and 80 pollen/m^3 was greater in Ourense (Tab. 1).

Figure 3 shows the model of behaviour throughout the day, calculated with the mean bihourly value of the 9 years under study [21]. A general model is presented since the behaviour pattern was fairly constant throughout the study period. The concentrations rise noticeably starting from 12 and 18 hours, representing 55% of total daily pollen during this time period. The maximum values took place at 15/16 in Santiago and with a slight delay in

Table 2. Correlation coefficients between *Alnus* pollen concentrations and meteorological parameters during the pollen season and prepeak period, considering the years altogether (Spearman's coef. *95%, **99.9% significance). Accumulated values of one and two preceding days of thermal oscillation, temperatures and sun hours are considered.

	Ourense		Santiago			Ourense		Santiago	
	Pollen season	Prepeak	Pollen season	Prepeak		Pollen season	Prepeak	Pollen season	Prepeak
Rainfall	-0.109*	-0.144*	-0.335**	-0.417**	Thermal Oscillation	-0.056	0.051	0.256**	0.377**
Humidity	0.081	-0.146*	-0.295**	-0.464**	Thermal-1	-0.078	0.004	0.271**	0.420**
Sun hours	-0.015	0.112	0.268**	0.418**	Thermal-2	-0.092*	-0.028	0.238**	0.373**
Sun-1	-0.046	0.115	0.246**	0.451**	T. maximum	-0.002	0.384**	0.337**	0.492**
Sun-2	-0.084	0.062	0.214**	0.427**	Max-1	-0.018	0.349**	0.343**	0.513**
					Max-2	-0.041	0.328**	0.310**	0.486**
Wind calm	-0.030	-0.176*	0.081	0.117	T. minimum	0.022	0.153*	-0.029	-0.061
Wind N-NE	-0.013	-0.083	0.118**	0.209**	Min-1	0.028	0.145*	-0.030	-0.081
Wind NE-S	0.145**	0.105	0.250**	0.263**	Min-2	0.029	0.152*	-0.020	-0.059
Wind S-SW	0.070	0.053	-0.225**	-0.331**	T. mean	-0.017	0.272**	0.181**	0.256**
Wind SW-N	-0.072	0.030	-0.125**	-0.081	Mean-1	-0.027	0.251**	0.187**	0.262**
Wind Path			-0.168**	-0.204**	Mean-2	-0.040	0.243**	0.180**	0.275**

Ourense. Starting from this moment, pollen concentrations decreased, being more marked in Santiago. In both cities there were minimum concentrations during the night.

Spearman's correlation test of the mean daily values was used to establish the relationship between pollen concentration and the main meteorological parameters. Table 2 summarises the results obtained, representing the correlation coefficient for both cities, taking into account the study period as a whole, and calculated with the data of the entire pollination period and the pre-peak period respectively. Correlations were significant in a large number of cases, especially in Santiago. In the case of Ourense, the significant correlations were obtained mainly for the pre-peak period. In both cities we found a positive correlation, with 99% significance with the maximum and average temperatures, as well as the total and pre-peak periods in Santiago, and only for the pre-peak period in Ourense. In Santiago, there was also significant correlation with daily thermal oscillation, hours of sunshine and when the wind blew from the north or north-east and north-east or south. Correlation was negative with 99% significance, in the case of Santiago, with rainfall, relative humidity and south or south-west and south-west or north winds.

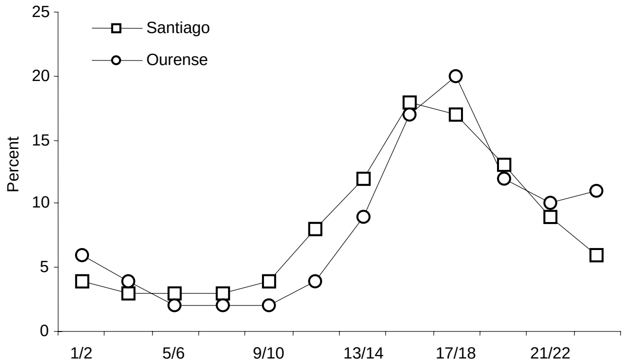

Figure 3. Hourly average pollen concentrations.

Finally, Scheffé's test was carried out to identify anomalous years altering the general prediction models to be obtained (Tab. 3). Temperature of the years under study presents an homogeneous behaviour during the (Alnus) pollen season, with the exception of 1998 and 2002 average temperature, but only in the case of the city of Ourense. However, analysis of the main meteorological parameters during the periods of chilling and heat accumulation prior to the onset of flowering (November-January), reveals significant differences in the case of

Table 3. Values of the Scheffé test coefficients (in bold 99.9% of significance) taking into account temperature average during pollen season (a) and temperature average during dormancy period (b), in Santiago (grey) and Ourense.

(a) Pollen season	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002
Ourense	8.442	8.092	10.211	8.775	9.108	11.967	8.176	9.880	10.198	4.383
Santiago	10.034	8.410	9.839	8.822	10.799	10.832	9.424	10.741	9.894	10.319
1993		0.291	1.000	0.643	0.967	0.967	0.997	0.994	1.000	1.000
1994	1.000		0.439	1.000	0.005	0.006	0.937	0.043	0.364	0.127
1995	0.316	0.118		0.805	0.833	0.841	1.000	0.958	1.000	0.999
1996	1.000	0.995	0.643		0.025	0.033	0.998	0.154	0.738	0.369
1997	0.998	0.960	0.949	1.000		1.000	0.565	1.000	0.871	0.999
1998	0.000	0.000	0.409	0.000	0.006		0.579	1.000	0.877	0.999
1999	1.000	1.000	0.194	0.998	0.981	0.000		0.795	1.000	0.966
2000	0.475	0.192	1.000	0.822	0.992	0.069	0.309		0.971	1.000
2001	0.263	0.088	1.000	0.593	0.942	0.334	0.157	1.000		1.000
2002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

(b) November-January	1992-93	1993-94	1994-95	1995-96	1996-97	1997-98	1998-99	1999-00	2000-01	2001-02
Ourense	5.314	9.530	10.257	11.228	9.330	11.179	8.259	7.884	10.870	6.232
Santiago	7.390	10.090	11.447	11.729	9.672	11.129	9.955	9.245	10.920	9.379
1992-93		0.879	0.351	0.243	0.957	0.484	0.909	0.989	0.599	0.983
1993-94	0.399		0.490	0.164	1.000	0.834	1.000	0.933	0.978	0.982
1994-95	0.157	0.998		1.000	0.134	1.000	0.318	0.005	0.999	0.019
1995-96	0.029	0.594	0.977		0.023	0.995	0.078	0.000	0.980	0.001
1996-97	0.470	1.000	0.982	0.367		0.408	1.000	1.000	0.777	1.000
1997-98	0.031	0.616	0.982	1.000	0.385		0.688	0.040	1.000	0.109
1998-99	0.871	0.926	0.352	0.012	0.971	0.013		0.977	0.937	0.996
1999-00	0.938	0.642	0.077	0.001	0.771	0.001	1.000		0.262	1.000
2000-01	0.060	0.871	0.999	1.000	0.703	1.000	0.064	0.006		0.431
2001-02	1.000	0.001	0.000	0.000	0.001	0.000	0.297	0.540	0.000	

Table 4. Santiago and Ourense Chill and Heat requirements, taking into account different threshold temperatures.

Santiago												
Threshold ºC	92-93	93-94	94-95	95-96	96-97	97-98	98-99	99-00	00-01	01-02	Mean	Coef. vs. %
chilling hours												
5	805	766	778	832	832		785	771		818	798	3.4
5.5	784	770	780	787	839		796	804		821	798	2.9
6	785	771	784	789	849		799	821		840	805	3.5
6.5	777	777	787	875	875	748	827	854	764	858	814	6.0
7	784	784	791	787	900	753	870	918	750	872	821	7.6
7.5	788	799	794	790	937	758	908	968	753	888	838	9.4
8	795	829	799	792	969	787	941	1022	767	918	862	10.6
9	836	872	848	832	1076	825	1047	1150	783	1050	932	14.3
10	938	1035	921	919	1148	916	1131	1084	855	1144	1009	11.1
11	1049	1103	1023	1036	1006	1033	1101	561	997	1132	1004	16.1
growth degree days												
6	26	44	38	59	72	72	19	63	27	71	49	42.5
6.5	24	42	35	56	69	68	17	58	25	63	46	42.8
7	22	39	33	52	65	64	15	53	23	55	42	43.4
7.5	20	37	30	49	62	60	13	48	21	47	39	44.3
8	18	34	70	45	58	56	11	43	19	39	39	48.8

Ourense												
ThresholdºC	92-93	93-94	94-95	95-96	96-97	97-98	98-99	99-00	00-01	01-02	Mean	Coef. vs. %
chilling hours												
5	795	815	837	812	793	774	872	819	640	958	812	9.8
5.5	797	819	840	812	798	774	878	822	655	967	816	9.6
6	809	807	845	819	802	762	908	832	664	987	824	10.3
6.5	815	842	850	826	821	763	929	875	670	1034	843	11.4
7	840	871	860	834	839	773	954	923	670	1069	863	12.3
7.5	859	889	873	840	858	780	978	957	663	1119	882	13.8
8	896	908	886	850	887	789	1037	993	656	1164	907	15.2
9	946	981	945	881	936	835	1134	1104	660	1194	962	16.2
10	1061	1067	1116	948	1015	916	1136	1153	621	1159	1019	16.0
11	1183	1145	1117	1052	1104	1001	998	1125	595	1061	1038	16.1
heat units												
6	20	55	45	57		59	70	41	49	71	52	30.2
6.5	16	51	41	54		56	64	35	46	63	47	32.2
7	11	47	36	51		52	57	29	43	55	42	35.1
7.5	7	43	32	48		49	51	23	40	47	38	39.2
8	2	39	27	45		45	44	17	37	38	33	45.1

2001–2002 average temperature but only in the case of Ourense. The rest of the parameters indicate that all of the years behaved similarly.

Predictive models. Once the atmospheric behaviour of this pollen had been identified, we tried to forecast its pollen-season timing and concentrations.

To predict the beginning of flowering, a thermal time model was used in order to quantify the chill requirement, and the growing degree days method in order to determine the heat requirement. To obtain the chilling accumulation expressed as "Chilling Hours", we established the start date as the moment when the mean temperature descends below the considered threshold temperature [31, 48]. These conditions generally take place during November, and the period of chilling accumulation continues until the second fortnight in December in both localities. The chilling requirements were similar, showing the smallest coefficient of standard deviation with a 5.5ºC threshold temperature. An average of 798 and 816 C.H. in Santiago and Ourense respectively were obtained (Tab. 4).

Once chilling requirements have been attained, heat accumulation begins and continues until the onset of flowering. Of the methods used to quantify the heat requirement, the sum of maximum temperatures has the smallest standard deviation coefficient in both localities. The smallest standard deviation was found with a

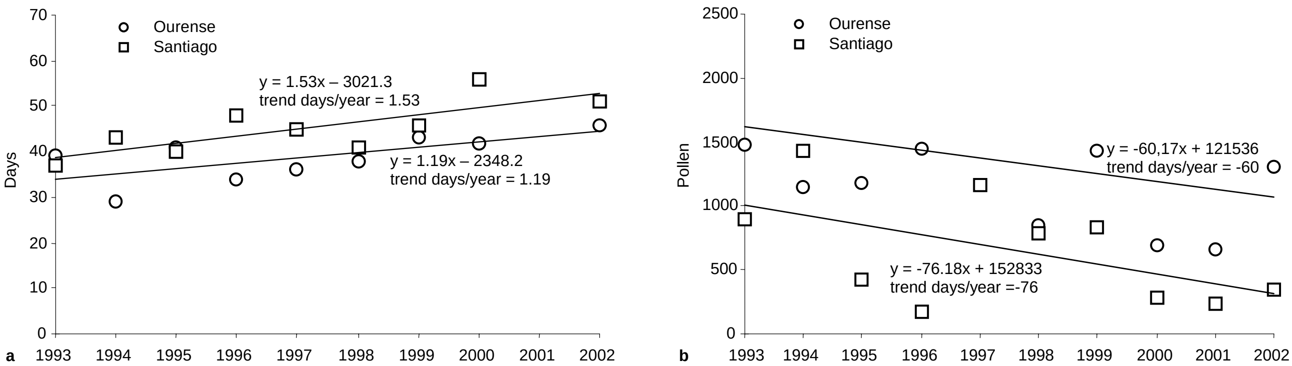

Figure 4. Trend line of the *Alnus* pollen season start dates (a) and annual total pollen (b). Data for 2001 were deleted in the first case as this was an anomalous year that can disguise the general tendency.

threshold temperature of 6ºC, and ca. 50 G.D.D. were required for flowering in both cities (Tab. 4).

In order to predict average daily *Alnus* concentrations, an analysis of linear regression was carried out, using as independent variable the parameters that had the greatest correlation with the pollen concentrations. Since the models that only take into account meteorological parameters do not explain a high percentage of variability (around 5-10%), previous day's pollen concentration was incorporated as dependent variable to improve them:

Ourense: *Alnus = 0.361 - (0.0297 × Rainfall) + (0.130 × Max. T.) + (0.3250 × Pollen),*

Santiago: *Alnus = 0.218 - (0.0021 × Rainfall) + (0.218 × Max. T.) + (0.8176 × Pollen).*

The regression equation that considers maximum temperature, rainfall and the previous day's pollen concentration explains the highest percentage of variability, 23% in Ourense and 69% in Santiago.

We tried to make a single model for both localities but this did not improve the proposals for each locality individually.

DISCUSSION

The pollen from *Alnus* is detected with important quantities in Spain in the atmosphere of the Euro-Siberian and Northwest regions. The beginning of the pollination period takes place earlier in the localities of the northern area with continental temperatures, such as in Ourense [35] or Ponferrada [51]. Figure 4a shows the start dates of the pollen season during the years under study, as well as the line indicating their trend. In spite of the recorded oscillations, the global trend has a positive slope, which indicates that the onset of the pollen season tended to be delayed gradually. The slope of the regression line indicates a delay of almost two days in Santiago and eight in Ourense, related with a low decrease trend of the mean temperature from November to January registered in both localities. There are some studies focused on the effect that the possible climatic change can influence the beginning of flowering. Most of them are based on spring-pollination species [11, 15, 25] where an opposite trend is showed: the onset of the pollen season tended to have begun progressively earlier in recent years. Frenguelli [19] points out a different behaviour when winter-flowering species and spring-flowering species were taken into account. The former tend to delay the beginning of the flowering period (as in our case), as opposed to the advance manifested by the latter.

The pollen indexes reached by *Alnus* in Ourense are higher than those of Santiago. These values are generally similar to those recorded in other points of north-west of Spain [1, 30, 35], León [17] or Extremadura [42], and localities in the north of Catalonia [8]. As we move away from the northern regions these values become smaller, recording a token amount of *Alnus* pollen in the coastal localities of southern Spain [43]. The highest values are generally produced during the second fortnight in January and the first fortnight in February. In the northern Europe, due to the cold climatological conditions, *Alnus* total pollen concentrations are higher. These values are produced slightly later, during the month of April [6, 29].

There were important variations in the total annual pollen index in both Ourense and Santiago. Some winter-spring flowering taxa, such as *Alnus*, experiment with biannual pollination rhythms due to an alternation in the mobilisation of nutrient reserves towards the tree's vegetative growth, or towards the reproduction structures [3, 46]. Although other studies [1] show behaviour patterns in which two years of low concentrations are followed by one of high values, no clear rhythm was found for the two studied cities. Also, the meteorological conditions in the north of Spain, with abundant precipitation, produce atmospheric cleansing that considerably reduces pollen concentrations in the air during the months when *Alnus* is in flower. This effect is more evident in Santiago where rainfall is more frequent and intense. Figure 4b shows the trend of total annual pollen values throughout the years under study, reflecting a decrease in the total number of *Alnus* pollen grains. The slope of the regression line indicates a decrease in the total number of pollen grains in the atmosphere of Ourense of 60 pollen grains per year, and 76 in Santiago, even though the low number of years considered in this study does not allow us to corroborate this trend.

In relation to the concentrations attained throughout the days, *Alnus* pollen displays a stable behaviour pattern with a maximum peak at 15/16 hours Santiago and 17/18 hours in Ourense (Fig. 3). The global behaviour pattern is very similar to the one described for nearby cities such as

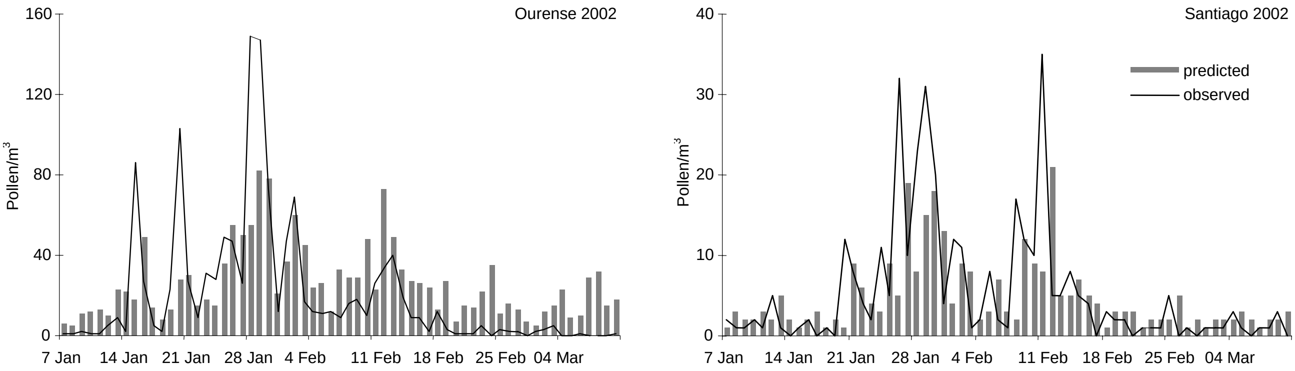

Figure 5. Predicted values (bars) and observed values (line) of *Alnus* pollen concentrations. Regression equations are also shown.

Vigo [46]. Studies carried out in the south of Spain reveal a maximum in the late evening [23], possibly due to the small number of pollens collected.

The influence that meteorological parameters, and in particular temperature, exerts on the presence of *Alnus* pollen in the atmosphere has been pointed out by different authors [1, 9, 20, 46]. The highest correlation coefficients were recorded with maximum and average temperature, together with daily thermal oscillation and rainfall in the case of Santiago.

These results reveal the effect of temperature on *Alnus* pollen levels. Therefore, temperature was used in both localities to establish models for both the onset of the pollen season and the pollen concentrations that may be attained. According to other authors [49], 40-50 days with temperature below 9.1ºC are needed to start *Alnus* flowering. An effective temperature for the termination of dormancy in many plants requiring chill is around 5ºC [39], although the range of temperatures between 2.5–9.1ºC are considered the most effective as chill units [20, 30, 44, 47]. The chilling accumulation period starts in November and continues until the first fortnight of January. The standard variation coefficients of the chilling requirements were low, 2.9% in Santiago and 9.6% in Ourense, with a threshold temperature of 5.5ºC. Previous papers [30] point out similar values for the same area by using different methods.

Final heat requirements to overcome dormancy period are satisfied by the tree in a short period of time, only 6–10 days once the chill has been accumulated. Of the different methods and threshold temperatures used and specified in material and methods, the lowest coefficient of standard deviation was obtained with maximum temperature in Santiago, and in Ourense with maximum temperature decreased by a threshold of 6ºC. Studies conducted in north-west Spain on spring-flowering species reveal good results using threshold temperatures of 5 or 5.5ºC [24, 30, 31, 47].

Verification of models. In order to determine the real validity of the models proposed, the data for the year 2002 was used, which was not taken into account in the model. According to the proposed model, once the chilling requirements have been attained, 52 heat units are needed for pollination in Ourense and 43 in Santiago. These requirements were reached on 11 January (while the real date of the start of pollination was 15 January) and 14 January (pollination took place on 20 January), respectively. Therefore, there are variations in the pollen season start dates compared to the real values, namely three days in the case of Ourense and six in that of Santiago. The cause of this delay could be the abundant rainfall, which considerably reduces pollen concentrations in the air during these days. This effect was more evident in Santiago, where rainfall was more intense, with a total of 43 mm during the days prior to pollination. When the rain stopped in both localities, the first grains were detected in the atmosphere, with some days of delay with regard to the estimated date. According to Scheffé's test (Tab. 3), 2002 differed from the other years under study in relation to pollen concentrations and temperatures from November, which may be another of the causes explaining such delays. Different authors point out that the shorter the period in which chilling is accumulated, the longer the required period of high temperatures [5, 32, 37, 44], which was also shown in studies carried out with this same taxon [30]. In this regard, the chilling accumulation period was shorter in 2002, finishing on 26 December. A greater number of heat units were therefore required in order to trigger flowering, thereby justifying the variations of the proposed models.

On verifying the proposed equations for predicting the daily mean pollen concentrations using the data of 2002, we observed that the curves follow the variations in the daily mean concentrations (Fig. 5).

CONCLUSIONS

The pollen of *Alnus* is present in the atmosphere of the localities studied during the winter months, mainly in January and February. During the years under study, a slight trend in the delay of the onset of the *Alnus* pollen season exists, contrary to the general trend of spring-flowering species. Further studies with a higher number of data could allow us to confirm this trend.

Throughout the day, the highest values are recorded at 15-18 hours. Temperature is the parameter that most influences the levels of *Alnus* pollen in the atmosphere. It was therefore chosen in the case of both localities to establish models predicting both the onset of the pollen season and the pollen concentrations that may be attained therein. In the first case, the estimated date according to the proposed models was a few days early (4-6) in relation to the real start date in 2002. The models for predicting the daily mean concentrations of *Alnus* pollen, using only meteorological variables as prediction variables, produce results with a low prediction level. This indicates that such variables alone do not explain its behaviour, and that other variables which better reflect the series of factors affecting the plant, and on which its pollen production and release depends, should be taken into account. In this regard, the autoregression with the previous days' pollen concentrations reflect this series of factors and therefore substantially improve the models' prediction capacity when included therein. Their main inconvenience is their low prediction horizon, since the data is not available until 24 hours prior to the day on which we want to predict pollen concentrations. Incorporating phenological data into the prediction models may improve pollen concentration predictions.

A shift in the timing of the alder pollen season is important since in north-west Spain this tree is located at the limit of its distribution. The variations detected in the beginning and intensity of its pollen season could help us to explain changes in its distribution, which is a very interesting tool for future studies of possible global climatic change.

Acknowledgements

This work was funded by the XUGA-PGDIT01PAT38301PR project. The authors would like to thank the Xunta de Galicia's Conselleria de Medio Ambiente.

REFERENCES

1. Aira MJ, Jato MV, Iglesias MI: *Alnus* and *Betula* pollen content in the atmosphere of Santiago de Compostela, North-West Spain (1993-1995). *Aerobiologia* 1998, **14**, 135-140.
2. Andersen TB: A model to predict the beginning of the pollen season. *Grana* 1991, **30**, 269-275.
3. Andersen ST: Influence of climatic variation on pollen season severity in wind-pollinated trees and herbs. *Grana* 1980, **19**, 47-52.
4. Arenas L, González C, Tabarés JM, Iglesias I, Jato V, Méndez J: Sensibilización cutánea a pólenes en pacientes afectos de rinoconjuntivitis-asma en la población de Ourense en el año 1994-1995. **In:** Aira MJ, Jato V, Iglesias (Eds): *Proceedings 1st European Symposium on Aerobiology, Santiago de Compostela, September 1996*, 93-94. Spain.
5. Aron R: Availability of chilling temperatures in California. *Agric Meteorol* 1983, 2, 351-363.
6. Atkinson H, Larsson K: A ten years record of the arboreal pollen in Stockholm, Sweden. *Grana* 1990, **29**, 229-237.
7. Belmonte J, Roure JM, March X: Aerobiology of Vigo, North-Western Spain: Atmospheric pollen spectrum and annual dynamics of the most important taxa and their clinical importance for allergy. *Aerobiologia* 1998, **14**, 155-163.
8. Belmonte J, Sbai L, Roure J: Aeropalinología en Catalunya: Estación de Girona (1999). *Rea* 2000, **6**, 87-90.
9. Brichi E, Frenguelli G, Mincigrucci G, Fornaciari M, Ferranti F, Romano B: Time linkages between pollination onsets of different taxa over an 11-year period in Perugia, Central Italy. *Aerobiologia* 1995, **11**, 57-61.
10. Carballeira A, Devesa C, Retuerto R, Santillan E, Ucieda E: *Bioclimatología de Galicia*. Fund. Barrié de la Maza, La Coruña 1983.
11. Corden J, Millington W: A study of Quercus pollen in the Derby area, UK. *Aerobiologia* 1999, **15**, 29-37.
12. Domínguez E, Galán C, Villamandos FE, Infante F: Handling and evaluation of the data from the aerobiological sampling. *Monografías Rea/Ean* 1992, **1**, 1-13.
13. Dopazo A: *Variación estacional y modelos predictivos de polen y esporas aeroalergénicos en Santiago de Compostela.* PhD Thesis, Universidad de Santiago de Compostela 2001.
14. Ekebom A, Vesterberg O, Hjelmroos M: Detection and quantification of airborne birch pollen allergens on PVDF membranes by immunoblotting and chemiluminiscence. *Grana* 1996, **35**, 113-118.
15. Emberlin J, Dentandt M, Gehrig R, Jäeger S, Nolard N, Rantio-Lehtimäki A: Responses in the start of *Betula* (birch) pollen seasons to recent changes in spring temperatures across Europe. *Int J Biometeorol* 2002, **46**, 159-170.
16. Faust M: *Physiology of temperate zone fruit trees.* JhonWiley & Sons, Toronto 1983.
17. Fernández-Gonzalez MD, Valencia RM, Vega A, Sagüés E: Aerobiología en Castilla y León (1995-1996): Estación de León. *Rea* 1998, **3**, 49-52.
18. Ferreiro M, Nuñez R, Rico M, Soto T, Lopez R: Pólenes alergénicos y polinosis en el área de La Coruña. *Rev Esp Alergol Inmunol Clin* 1998, **13**, 98-101.
19. Frenguelli G: Interactions between climatic changes and allergenic plants. *Monaldi Arch Chest Dis* 2002, **57(2)**, 141-143.
20. Frenguelli G, Spieksma F, Bricchi E, Romano B, Mincigrucci G, Nikkels AH, Dankaart W, Ferranti F: The influence of air temperature on the starting dates of the pollen season of *Alnus* and *Populus*. *Grana* 1991, **30**, 196-200.
21. Galán C, Tormo R, Cuevas J, Infante F, Domínguez E: Theoretical daily variation patterns of airborne pollen in the South-west of Spain. *Grana* 1991, **30**, 201-209.
22. Galán C, Emberlin J, Domínguez E, Bryant R, Villamandos F: A comparative analysis of daily variations in the Graminae pollen counts at Córdoba, Spain and London, UK. *Grana* 1995, **34**, 189-198.
23. Galán C, García-Mozo H, Cariñanos P, Alcázar P, Domínguez-Vilches E: The role of temperature in the onset of the *Olea europaea* L. pollen season in southwestern Spain. *Int J Biometeorol* 2001, **45**, 8-12.
24. García-Mozo H, Galán C, Gomez-Casero MT, Domínguez-Vilches E: A comparative study of different temperature accumulation methods for predicting the start of the *Quercus* pollen season in Córdoba (South West Spain). *Grana* 2000, **39**, 194-199.
25. Garcia-Mozo H, Galán C, Aira MJ, Belmonte J, Díaz de la Guardia C, Fernandez D, Gutierrez AM, Rodriguez FJ, Trigo MM, Dominguez-Vilches E: Modelling start oak pollen season in different climatic zones in Spain. *Agric Forest Meteorol* 2002, **110**, 247-257.
26. Hayek B, Vangelista L, Pastore A, Sperr W, Valenta P, Vrtala S, Niederberger V, Twardosz A, Kraft D, Valenta R: Molecular and immunologic characterization of a highly cross-reactive two EF-hand calcium-binding alder pollen allergen, Aln g 4: structural basis for calcium modulated Ig-E recognition. *J Immunol* 1998, **161**, 7031-9.
27. Hirst JM: An automatic volumetric spore-trap. *Ann Appl Biol* 1952, **36**, 257-265.
28. Jäger S: Plant Identification, Taxonomy, Allergic Cross-Reactions... **In**: Spieksma FTh, Domínguez E, Frenguelli G, Emberlin J, Jäger S (Eds): *Course Assesment of airborne pollen concentrations 1995*. Leiden (Holland).
29. Jäger S, Nilsson S, Berggren B, Pessi A, Helander M, Ramfjord H: Trends of some airborne tree pollen in the Nordic countries and Austria, 1980-1993. A comparison between Stockholm, Trondheim, Turku and Wien. *Grana* 1996, **35**, 171-178.
30. Jato V, Frenguelli G, Rodríguez-Rajo FJ, Aira MJ: Temperature requirements of *Alnus* pollen in Spain and Italy (1994-1998). *Grana* 2000, **39**, 240-245.
31. Jato V, Rodríguez-Rajo FJ, Méndez J, Aira MJ: Phenological behaviour of Quercus in Ourense (N.W. Spain) and its relationship with the atmospheric pollen season. *Int J Biometeorol* 2002, **46**, 176-184.
32. Landsberg JJ: Apple fruit bud development and growth; analysis and an empirical model. *Ann Bot* 1974, **38**, 1013-1023.
33. Ljungkvist S, Bringfelt B, Fredriksson V: Correlation between the pollen content of the Stockholm air and metereological data. *Grana* 1977, **16**, 145-146.

34. Martínez-Cortizas A, Pérez-Alberti A: *Atlas climático de Galicia*. Xunta de Galicia, Santiago de Compostela 1999.

35. Mendez J: *Modelos de comportamiento estacional e intradiurno de pólenes y esporas de la ciudad de Ourense y su relación con los parámetros meteorológicos.* PhD Thesis. Universidad de Vigo 2000.

36. Montero JL, González JL: *Diagramas bioclimáticos.* Instituto Nacional para la conservación de la Naturaleza, Madrid 1983.

37. Myking T: Dormancy, budburst and impacts of climatic warming in coastal-inland and altitudinal *Betula pendula* and *B. pubescens* ecotypes. **In:** Lieth H and Schwartz MD (Eds): *Phenology in seasonal climates I*, 51-66. Backhuys Publ., Leiden, The Netherlands 1997.

38. Nilsson S, Persson S: Tree pollen spectra in the Stockholm region (Sweden), 1973-1980. *Grana* 1981, **20**, 179-182.

39. Nooden L, Weber J: Environmental and hormonal control of dormancy in terminal buds of plants. **In:** Clutter ME (Eds): *Dormancy and development arrest*, 220-260. Academic Press, New York, USA 1978.

40. Norris-Hill J: The modelling of daily Poaceae pollen concentrations. *Grana* 1995, **34**, 182-188.

41. Norris-Hill J, Emberlin J: Diurnal variations of pollen concentration in the air of north-central London. *Grana* 1991, **30**, 229-234.

42. Paulino R, Tormo R, Silva I, Muñóz A: Aeropalinología en Andalucía: Estación de Málaga (2000-2001). *Rea* 2000, **7**, 83-88.

43. Recio M, Trigo M, Docampo S, Cabezudo B: Aeropalinología en Extremadura: Estación de Cáceres (1999). *Rea* 2002, **6**, 107-110.

44. Richardson EA, Schuyler DS, Walker DW: A model for estimating the completion of rest of "Redhaven" and "Elberta" peach trees. *Hortoscience* 1974, **9**, 331-332.

45. Rivas Martínez S: *Memoria del mapa de series de vegetación de España*. Serie Técnica ICONA, Madrid 1987.

46. Rodríguez-Rajo FJ: *El polen como fuente de contaminación ambiental en la ciudad de Vigo*. PhD Thesis. University of Vigo 2000.

47. Rodríguez-Rajo FJ, Méndez J, Jato MV: Influencia de la temperatura en la floración de Quercus en el sur de Galicia (Ourense y Vigo, 1994-98). *Acta Bot Malac* 2000, **25**, 153-163.

48. Rodríguez-Rajo FJ, Frenguelli G, Jato MV: Effect of air temperature on forecasting the start of the *Betula* pollen season at two contrasting sites in the South of Europe. *Int J Biometeorol* 2003, **47**, 117-125.

49. Spieksma F, Frenguelli G, Nikkels AH, Mincigrucci G, Smithuis L, Bricchi E, Dankaart W, Romano B: Comparative study of airborne pollen concentrations in central Italy and The Netherlands (1982-1985). *Grana* 1989, **28**, 25-36.

50. Spieksma F, Nolard N, Frenguelli G, Van Moerbeke D: *Polen atmosférico en Europa*. UCB, Bruselas 1993.

51. Valencia-Barrera R, Vega A, Fernández-González D, Mencía J, Díaz C: Aeropalinología en Castilla y León: Estación de Ponferrada (1998). *Rea* 2000, **6**, 71-74.

52. Wareing P, Phillips I: *The control of growth and differentiation in plants*. Pergamon Press, Oxford 1978.